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(57) Abstract: The present invention relates to a method of evaluating the efficiency of sterilization processes by measurement of degradation level of prion protein indicators. When exposed to sterilization conditions, prion indicators are degraded in a manner proportionally indicate the level of degradation of prion proteins themselves on medical devices or other surfaces usable in surgery and health care.

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R.N. ROSENBERG ET AL.: "Precautions in handling tissues, fluids and other contaminated materials from patients with documented or suspected Creutzfeldt-Jakob disease" ANNALES OF NEUROLOGY, vol. 19, no. 1, 1986, pages 75-77, XP000923098 BOSTON MA USA cited in the application table 1	1-13
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* Special categories of cited documents: "A" documents defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "S" document member of the same patent family		
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(54) Title: BIOLOGICAL INDICATORS FOR VALIDATING A PRION STERILIZATION PROCESS

(54) Titre: INDICATEURS BIOLOGIQUES DESTINES A VALIDER UNE PROCEDURE DE STERILISATION DIRIGEE CONTRE
LES PRIONS

(57) Abstract

The present invention relates to a method of evaluating the efficiency of sterilization processes by measurement of degradation level of prion protein indicators. When exposed to sterilization conditions, prion indicators are degraded in a manner to proportionally indicate the level of degradation of prion proteins themselves on medical devices or other surfaces usable in surgery and health cares.

(57) Abrégé

L'invention concerne un procédé pour évaluer l'efficacité des procédures de stérilisation par la mesure du taux de dégradation des indicateurs des protéines de prions. Exposés à ces conditions, les indicateurs des protéines de prions se décomposent d'une manière qui indique de façon proportionnelle le taux de décomposition des protéines de prions proprement dites à la surface de dispositifs médicaux ou d'autres surfaces utilisées en chirurgie et en soins médicaux.

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(54) Title: BIOLOGICAL INDICATORS FOR VALIDATING A PRION STERILIZATION PROCESS (57) Abstract <p>The present invention relates to a method of evaluating the efficiency of sterilization processes by measurement of degradation level of prion protein indicators. When exposed to sterilization conditions, prion indicators are degraded in a manner to proportionally indicate the level of degradation of prion proteins themselves on medical devices or other surfaces usable in surgery and health cares.</p>		

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BIOLOGICAL INDICATORS FOR VALIDATING A PRION
STERILIZATION PROCESS

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to a method of control of sterilization process, which comprises the steps of measuring the level of degradation of a prion protein degradation indicator when exposed to different sterilization processes.

(b) Description of Prior Art

Biological indicators are considered essential to evaluate the efficacy of any sterilization procedure since chemical and physical monitors are not completely reliable. The latter are useful for detecting gross sterilization, but spore tests are absolutely required for any assurance of sterilization since they are more resistant to heat than viruses and vegetative bacteria. The biological indicators are usually composed of bacterial spores of *Bacillus stearothermophilus* (for autoclaves and chemical vapor sterilizers) or *Bacillus subtilis* (for dry heat and ethylene oxide sterilizers) which are removed after sterilization treatment and incubated at the appropriate temperature to observe any microbial growth (Dental Products Report, October 1995, pp. 96-104). However, in this day and age, bacterial spores are no longer the most resistant life forms since the discovery of prions.

Sup35 protein (hereby referred to as Sup35p) carrying [PSI+] is a prion-like protein due to its striking similarities to prions. Indeed, the N-terminal of Sup35p is insoluble in non-ionic detergents and partly resistant to proteases' action. In addition, it principally forms abnormal amyloid filaments composed

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mainly of β -sheets, as opposed to the normal isoform of the protein mostly formed of α -helices (Glover, J.R., Kowal, A.S., Et al. *Cell* (1997) 89:811-819; King, C., Tittmann, P. et al. *Proc. Natl. Acad. Sci. USA* (1997) 94:6619-6622).

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The intracellular accumulation of these abnormal prion filaments is responsible for inducing transmissible spongiform encephalopathies in both animals and humans, hence the importance of degrading the filaments in order to prevent any iatrogenic transmission of the disease. Several cases of iatrogenic contamination have been reported due to the utilization of contaminated medical equipment, such as EEG electrodes, which had been previously in contact with Creutzfeld-Jakob patients and inadequately sterilized (Jarvis, W.R. *Hospital Infection Control* (1985) 12 (12):145-148). Since there also remains the possibility of blood contamination, which has not yet been ruled out, most medical instruments enter in the category of being at risk of being contaminated but that, at different levels depending on the case history of the patient.

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The unavailability of sterilization indicators to attest of prion degradation renders the devices inadequate and even dangerous for multiple usage. As of today, most countries have adopted similar requirements for sterilization of contaminated instruments. The recommended procedures for sterilization of medical instruments used on patients at high risk is the incineration of any disposable equipment that has been in contact with a patient or, at the very least, soaking in 1N sodium hydroxide, which is very corrosive for metallic instruments, or autoclaving at 132°C/1atm

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5 pressure for an hour (Rosenberg, R.N. et al., *Annals of*
10 *Neurology* (1986) 19(1):75-77; Galtier, F., *J. Pharm.*
Clin. (1994) 13 :317-9) which can deform
thermosensitive materials such as polymers.

5 It would be highly desirable to be provided with
a novel indicator of prion degradation and therefore,
15 of complete sterilization of medical devices.

SUMMARY OF THE INVENTION

10 The solution therefore lies in the development
20 of this novel sterilization indicator, based on Sup35
protein, which would insure that all the medical
devices are thoroughly sterilized and fit for
25 utilization by proving the degradation of prions. This
15 indicator could be used for any sterilization process
commonly used, as well as novel techniques such as low-
temperature plasma gas or ozone-based sterilizers for
instance.

30 One aim of the present invention is to provide a
20 novel indicator of prion degradation and therefore, of
complete sterilization of medical devices.

35 In accordance with the present invention there
is provided a method of evaluating the efficiency of a
sterilization process, which comprises the steps of:

25 a) subjecting a sufficient amount of at least
40 one prion protein degradation indicator in a container
to the sterilization process; and

b) determining the level of degradation of the
indicator.

45 30 An aspect of the invention is that the indicator
may be transcribed by a gene naturally occurring in a
fungus, most particularly in *Saccharomyces cerevisiae*,
or *Podospora anserina*.
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5 The indicator may be transcribed by gene
selected from the group consisting of SUP35, URE2, and
10 HET-s.

The indicator may be selected from the group
5 consisting of Sup35p, Ure2p, Het-s protein, and
combination thereof.

15 The indicator may be a purified form naturally
occurring in fungi, a recombinant form, an analog, a
mutant, or a fragment of the indicator.

20 The indicator may be a biological indicator,
biochemical indicator, or chemical indicator.

Of particular aspects of the invention, the
measurement of indicator degradation may be performed
by determining the weight or the mass, quantifying
25 radicals, colorimetric variations, radiometry,
nephelometry, immuno-enzymatic method, Western
blotting, dot blotting, radioimmuno assay, circular
dichroism, electron microscopy, fluorescent microscopy,
30 FTIR, Congo red binding, or proteinase digestion.

20 The sterilization process may be performed by
autoclaving, chemical exposure, dry heating, low
temperature plasma gas, ozone-based exposure, or
35 sterilization techniques using alkylant and/or
oxidizing sterilizing agents.

25 The chemical exposure may be a vapor or a
solution selected from the group consisting of
40 detergent, ethylene oxide, protease, sodium hydroxide,
and enzyme.

The amount of indicator exposed to sterilization
45 30 processes may be between 0.1 ng to 100 g.

The container may be of a material selected from
the group consisting of paper, glass, borosilicate,
50 metal, polymer, alloy and composite.

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The container may also be porous, permeable, or semi-permeable.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the production of bacterially-expressed recombinant SUP35 N-terminal segment;

Fig. 2 illustrates the transmission electron microscopy of recombinant Sup35 N-terminal segment in different solutions;

Fig. 3 illustrates circular dichroism analysis of Sup35 N-terminal protein;

Fig. 4 illustrates the effect of autoclave and ethylene oxide treatments on Sup35 N-segment protein integrity; and

Fig. 5 illustrates the effect of Sterrad® 100 treatment on Sup35 N-segment integrity.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one embodiment of the present invention, there is provided a novel indicator of prion degradation and therefore, of complete sterilization of medical devices.

A particular aspect of the present invention is the use of [PSI+], a non-mendelian genetic factor encoded by the SUP35 gene of the budding yeast *Saccharomyces cerevisiae*, and Het-s encoded by *Podospora anserina*, as indicator of prions protein degradation in sterilization of medical devices and all other apparatus, surfaces, or things used in surgical procedures and health cares.

Another embodiment of the invention is the use of fertilisation indicators formed of Sup35p, Ure2p,

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5 Her-s, or Psi that is quite simple to use. The
indicator may be in solution within a glass vial, so
10 that the container would be resistant to any
sterilization technique, whether it is autoclaved using
5 high heat and pressure or low-temperature techniques
such as plasma. Non-denaturing buffers such as
15 Tris/EDTA, TFA/acetonitrile, or even 2M urea would be
used in order to maintain the integrity of the Sup35p
filaments.

10 It is known that a single infectious unit of
prion corresponds to 10^4 - 10^5 PrP molecules, or 0.5-5 fg,
20 which is below the detection limit of SDS-PAGE gels
(Hill, A.F., Antoniou, M. and Collinge, J., *Journal of*
General Virology (1999) 80:11-14).

25 Hence, in an other embodiment of the invention,
there is a proof of degradation of a larger amount of
protein, such as 10µg, that insure that complete
30 sterilization has occurred and therefore, that the
medical instrument is safe for reuse. This is based on
20 the consideration that if there is a structural
modification of the protein, i.e. if the protein
undergoes a change in conformation or degradation
35 following exposure to the various sterilization
techniques, it is rendered inactive and therefore, non-
25 infectious.

40 Moreover, by using 10µg of indicator, the
invention allows to be able to easily detect any
degradation of the protein by SDS-PAGE gels stained
with Coomassie Brilliant Blue for example, a common
45 30 laboratory technique, since as little as 0.1µg of
protein can be detected by this method (Sambrook, J.,
Fritsch, E.F., and Maniatis, T. *Molecular Cloning*, a

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laboratory manual. Cold Spring Harbor Laboratory Press, 2nd edition, 1989).

In an other embodiment of the invention, degradation or alteration can also be estimated by Western Blot or dot blot using an antibody against the tagged protein to estimate the lack of or a modified detection signal being generated by any alteration of the target Sup35 protein. In addition, the indicator degradation could also be detected by color change of the solution, which would confirm sterilization. If needed, techniques such as circular dichroism, electron microscopy, fluorescent microscopy, FTIR, Congo Red binding or proteinase K digestion could also be used to detect the change in conformation of the sterilized protein from β -sheets to α -helices, thus displaying the degradation of the protein, and therefore, its inactivation.

Since the materials used for the indicator (glass vials, solutions, etc.) are quite common and inexpensive, the total cost of production of such an indicator is reasonably low. It therefore renders it very affordable for any institution, hospital or industry that would purchase it to ensure the safety of their medical instruments.

In an embodiment the invention, the sterilization indicator is also cost effective since all instruments can be tested and may be proven safe for reuse, if and only if the sterilization indicator demonstrate complete inactivation following an entire cycle of sterilization. Common spore tests do not rule out completely the possibility that active residual proteins do remain on the surface of the devices. Moreover, these techniques can alter the quality of the

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5 instruments, hence the quality of the medical care
provided. Replacing all hypothetically contaminated
10 instruments would indeed be very costly for medical
services and reusable instruments might be discarded in
5 the process for fear of contamination.

15 MATERIALS AND METHODS

Bacterial strains

For cloning experiments, the *Escherichia coli*
10 SURE strain was routinely used (InVitrogen™). For
protein expression and purification, expression plasmid
20 was transformed into the *E. coli* BL21(pRep4) strain
(Novagen).

DNA manipulations and protein purification

25 Standard DNA techniques have been described
before (Sambrook et al., 1989). DNA sequencing was
performed at Institut Armand Frappier DNA sequencing
30 facilities (Montreal, Canada). Protein
expression/purification procedures were performed as
20 described by the manufacturer (Clontech).

35 Cloning of the aggregating N-terminal domain of Sup35 in a bacterial expression vector

The first 759bp region of Sup35 encoding the
peptidic region sufficient for aggregation was PCR
25 amplified from a genomic clone in pEMBLyex4 kindly
provided by Dr. Ter-Avanesyan (Moscow, Russia; (Glover.
40 J.R., Kowal, A.S., Et al. Cell (1997) 89: 811-819). The
following primers were used: (a) 5'-
AGTGGATCCTCGGATTCAAACCAAGGCAA-3' (introducing a BamHI
45 restriction enzyme site, underlined), and (b) 5'-
30 CGCGTCGACATCGTTAACACCTCCGTC-3' (introducing a SalI
restriction enzyme site, underlined). The fragment was
then cloned into p77Blue3 (Perfectly Blunt Cloning Kit

5 - Novagen) into the SURE *E. coli* strain (InVitrogen).
Positive clones were sequenced to assess any mutation
10 or deletion in the gene. The Sup35 gene N-segment was
then excised with BamHI and SalI and inserted into the
5 expression vector pQE30 (Qiagen) using the same
restriction sites. Positive clones in pQE30 were the
15 transferred in BL21[pREP4] for protein expression and
purification.

Protein expression and purification

10 Protocols were performed mostly according to the
20 manufacturers. Induction of a 1L of bacterial culture
(OD₆₀₀ of 0.8) was done using IPTG (final concentration
of 1mM) for an hour at room temperature. The cells were
harvested, resuspended in 50ml of Buffer B (8M urea,
25 0.1M Na-phosphate, 0.01M Tris HCl pH 8.0), sonicated
15 and centrifuged at 10 000xg for 15 min at 4°C. The
supernatant was collected and loaded onto a Ni²⁺-NTA
column (TALON metal affinity resin; Clontech) for
30 affinity chromatography using a pH gradient with the
20 denaturing 8M urea purification protocol from Qiagen.
The samples were analyzed by SDS-PAGE (gel
35 electrophoresis technique used to estimate the size and
amount of the protein) and by Western Blot using a
mouse anti-histidine antibody (Qiagen) against the 6-
25 histidine tail present in the pQE30 vector, in order to
40 specifically detect the protein. After separation of
the proteins on SDS-PAGE and electroblotting onto
nitrocellulose, the membrane was incubated with the
primary antibody (anti-HIS RGS, from Qiagen, 1:2000) in
45 30 10 mM Tris pH 7.5, 100 mM NaCl (TBS) with 5% Non fat
milk, 0.1% Tween™20 for an hour. There was then 3
washes (10 minutes each) with TBS 0.1% Tween™20.
50 Detection was performed with the BM Chemiluminescence

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Kit using an anti-mouse antibody coupled to horseradish peroxidase (1:4000) (Roche Diagnostics). Membranes were finally exposed onto radiographic films and developed.

Filament induction and analysis

To induce the formation of the filaments, a 6h to 12h dialysis at room temperature of the protein (9 μ M, in 8M urea solution) against either a 2M urea, 30mM Tris-HCl pH 8.0, 300mM NaCl (referred to as "2M urea") solution or 0.1% Trifluoroacetic acid, 40% acetonitrile solution (referred to as "TA") or a Tris/EDTA solution (10 mM Tris pH 8.0 EDTA 1mM, "TE") was performed.

Transmission Electron Microscopy (TEM)

Samples (50 μ l) of the filament suspension were sedimented by ultracentrifugation (178000g, 20min, Beckman Airfuge) onto a carbon-formvar coated copper grids (3mm diameter, 200 mesh). These grids were then negatively stained by 3% (wt/vol) PTA (Phosphotungstic acid) and by 2% (wt/vol) uranyl acetate for 1 minute each. The samples were then observed using a Transmission Electron Microscope Hitachi H-7100 at 75 kV.

Circular Dichroism (CD) Spectroscopy

CD spectra of a 9 μ M filament suspension (2M urea) were recorded on a Jasco J710 Spectropolarimeter at room temperature using a 0.05cm pathlength cell. Samples were scanned with the following settings: scan speed: 100nm/min; response time: 0.25 sec; accumulations: 3 (empty cell), 5 (buffer alone) and 10 (protein samples); sensitivity: 50mdeg; starting wavelength: 260nm; ending wavelength: 200nm.

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Sterilization assays

Autoclaving at 121°C for an hour and Sterivac® (ethylene oxide) was used as negative controls; Sterrad® 100, which uses a combination of hydrogen peroxide and gas plasma as sterilizing agents were used as the experimental processes. The samples were submitted to either one entire cycle of each process or to only a quarter of a cycle, as it was the case with ozone. Following this, the degradation of Sup35 was evaluated. 10 µg of protein was exposed to the sterilization processes described in Table 1. Degradation of the protein was assayed by SDS-PAGE using Silver Nitrate and Coomassie Blue coloration as well as TEM micrographs (filament formation). Immunological detection using chemiluminescence (described above) was also used, following the sterilization processes.

Table 1

Sterilization cycles used for the evaluation of the degradation of Sup35p

Sterilization process	Sterilizing agent	Cycle	Time required for complete cycle
Autoclave	Heat	Temperature: 121°C Pressure: 1 atm	1 hour
Sterivac®, 3M	Ethylene Oxide (EO)	(a) Temperature: 134°F (b) Preheating time: 30 min. (c) Sterilization time: 2h10 (d) Ventilation time: 12h	Approx. 16 hours
Sterrad® 100, Johnson and Johnson	H ₂ O ₂ and gas plasma	(a) vacuum (0.3 torr): 5-20min (b) injection of H ₂ O ₂ 58% + H ₂ O : 6min (c) diffusion (0.5 torr): 44min (d) plasma: 15min	Approx. 75-95 min.

RESULTS

Purification and Characterization of SUP35 N-protein

The SUP35 gene encodes a 76.5 kDa ribosome-associated protein. However, it has been shown that only the first 114 amino acids are sufficient for filament formation (King, C., Tittmann, P. et al. *Proc. Natl. Acad. Sci. USA* (1997) 94:6618-6622). DNA primers similar to those already described (Glover, J.R., Kowal, A.S., Et al. *Cell* (1997) 89:811-819) were used to amplify the first 639 nucleotides including and from the initiation codon, using a genomic clone provided by Dr. Ter-Avanesyan. Glover et al. (1997) has shown that the resulting 213 amino acid long peptide could exhibit many biochemical features similar to prions. The expressed protein, purified under denaturing conditions, has an apparent molecular weight of 30 kDa, as estimated by SDS-PAGE analysis (Fig. 1, left-hand panel). Bacterially-expressed protein from purified through nickel chromatography (Materials and Methods) and protein samples were analyzed by SDS-PAGE and Coomassie Blue staining (left-hand panel; and Western blotting with an antibody against the 6XHIS tag (right-hand panel). Identity of the protein observed by Coomassie staining of the gel was confirmed, using and antibody raised against the 6XHIS tag, which is present in the bacterially-expressed protein due to its incorporation in frame at the N-terminus of the peptide (Fig. 1, right-hand panel).

To ensure that the purified SUP35 N-protein was behaving similarly to prions, the ability to undergo ordered aggregation was investigated, forming amyloid-like filaments. Those can be observed by transmission electronic microscopy (TEM). TEM images of protein

5 suspension in 8M urea or slowly dialyzed against 2M
urea or trifluoroacetic acid/acetonitrile 0.1%/40% (TE)
10 solution and maintained at 4°C for a week are shown in
Fig. 2. Bacterially-produced protein in 8M urea was
5 dialyzed against either 2M urea, trifluoroacetic
acid/acetonitrile 0.1%/40% (TA) or Tris-EDTA (TE),
15 maintained for one week at 4°C and processed for TEM.
(M refers to the marker). Indeed, the Sup35 protein
unless in 8M urea solution (even for weeks at 4°C) tend
10 to form aggregates easily observed by TEM analysis.

20 Moreover, extensive aging of the solutions
containing Sup35p should exhibit β sheet-like
characteristics, with a single differential absorption
minimum near 220 nm when analyzed by circular
25 dichroism. As it can be seen in Fig. 3, it is possible
15 to distinguish a spreading of the peak of protein in 2M
urea (ordered aggregates) from the random coiling of
Sup35p in the 8M urea solution.

30 From these results, it is concluded that the
20 bacterially-expressed N-portion of the Sup35 protein
behaves as expected and exhibits many biochemical
features resembling to prions.

35 Sup35p Stability to Various Sterilizing Process

The efficacy of sterilizing treatments was
25 assessed based on their impact on the integrity of the
Sup35p. Samples of the Sup35 protein, kept under
40 different forms, were processed and then analyzed by
SDS-PAGE and/or Western blotting.

45 The Applicants are the first to confirm that
30 classical autoclave sterilization cycle was unable to
destroy Sup35 protein as it is the case for prions. No
intact protein could be recovered from Sup35p kept in
50 8M urea (no aggregates) after autoclave while filaments

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5 from the Sup35 protein in TFA were resistant to
degradation, as seen from Coomassie staining of the
10 SDS-PAGE (Fig. 4, top panel, Sup35 protein in 8M urea
or in TA was processed for sterilization and then
5 analyzed for integrity by SDS-PAGE. (U refers to
untreated and T, to treated samples)). Similar results
15 were obtained when the same samples were exposed to
ethylene oxide (Fig. 4, bottom panel). From these
results, it is conclude that autoclave and ethylene
10 oxide treatments are unable to degrade the orderly
aggregated Sup35 protein.

20 On the other hand, the 8M urea and 2M urea
proteins were degraded upon treatment to the Sterrad®
100 treatment (oxidative process, combining hydrogen
25 peroxide and gas plasma). Aggregates of the 2M urea
protein could be destroyed by this treatment.
Aggregates of the TA protein could however resist to
the sterilizing process, as evaluated by the intact
30 protein seen in the Coomassie Blue stained gel (Fig. 5,
top panel, Sup35 protein samples in 8M urea, 2M urea or
20 trifluoroacetic acid/acetonitrile 0.1%/40% (TA) were
processed and the remaining intact protein was analyzed
35 by SDS-PAGE revealed by Coomassie Blue staining (top
panel) or Western blot analysis, with an antibody
25 against 6XHIS tag (bottom panel). (U refers to
untreated, T to treated samples, and * in Western blot
40 panel to intact Sup35p). To increase the sensitivity of
the detection technique and to ensure that this
treatment could indeed degrade the filaments of the 2M
45 urea Sup35 protein, Western blot analysis was performed
30 using an antibody detecting the 6XHIS tag present at
the N-terminus of the bacterially-expressed protein
(Fig. 5, bottom panel). These experiments show that the

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5 Sterrad® 100 process can degrade the aggregates of the
2M urea kept Sup35 protein. The only resistance
10 observed was in the samples of the Sup35 placed in TA.
There are two possible explanations for these
5 unexpected results. First, there could have been an
interaction between the Trifluoroacetic
15 acid/acetonitrile and the hydrogen peroxide used as the
sterilizing agent in Sterrad systems, which could have
inhibited the oxidative potential of this process. This
10 increased resistance could also have been caused by the
protonation of the protein by TA solution, which would
20 render the protein less susceptible to oxidative effect
of hydrogen peroxide. TEM analyses of samples in
different solutions for sterilization procedures used
25 in this study indicated desintegrity of Sup35 filament
conformation. These observations confirmed the results
obtained by other methods, such as Coomassie Blue and
Western Blot as described here-above.

30 From these results, it is expected that other
sterilization techniques which use oxidative
20 sterilizing agents, such as ozone-, peracetic acid-
based sterilizers, etc. would also be efficient to
alter Sup35 protein.
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While the invention has been described in con-
25 nection with specific embodiments thereof, it will be
understood that it is capable of further modifications
40 and this application is intended to cover any varia-
tions, uses, or adaptations of the invention following,
in general, the principles of the invention and
30 including such departures from the present disclosure
as come within known or customary practice within the
art to which the invention pertains and as may be
applied to the essential features hereinbefore set
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forth, and as follows in the scope of the appended
claims.

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WHAT IS CLAIMED IS:

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1. A method of evaluating the efficiency of a sterilization process, which comprises the steps of:

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a) subjecting a sufficient amount of at least one prion protein degradation indicator in a container to said sterilization process; and

b) determining the level of degradation of said indicator.

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2. The method according to claim 1, wherein said indicator of step a) is transcribed by a gene naturally occurring in a fungus.

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3. The method according to claim 2, wherein said fungus is selected from the group consisting of *Saccharomyces cerevisiae*, and *Podospora anserina*.

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4. The method according to claim 3, wherein said indicator is transcribed by a gene selected from the group consisting of SUP35, URE2, and HET-s.

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5. The method according to claim 2, wherein said indicator is selected from the group consisting of Sup35p, Ure2p, Het-s protein, and combination thereof.

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6. The method according to claims 1 to 5, wherein said indicator is a purified form naturally occurring in *Saccharomyces cerevisiae*, *Podospora anserina* or a fungus, a recombinant form, an analog, a mutant, or a fragment of said indicator.

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7. The method according to claim 1 to 5, wherein said indicator is a biological indicator, a biochemical indicator, or a chemical indicator.

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8. The method according to claim 1, wherein step b) is performed by determining the weight or the mass, quantifying radicals, colorimetric variations, radiometry, nephelometry, immuno-enzymatic method, Western blotting, dot blotting, radioimmuno assay, circular dichroism, electron microscopy, fluorescent microscopy, FTIR, Congo red binding, or proteinase digestion.

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9. The method according to claim 1, wherein said sterilization process is performed by autoclaving, chemical exposure, dry heating, low temperature plasma gas, ozone-based exposure, or sterilization techniques using alkylant and/or oxidizing sterilizing agents.

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10. The method according to claim 9, wherein said chemical exposure is a vapor or a solution selected from the group consisting of detergent, ethylene oxide, protease, sodium hydroxide, and enzyme.

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11. The method of claim 1, wherein said amount of indicator of step a) is between 0.1 ng to 100 g.

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12. The method of claim 1, wherein said container is of a material selected from the group consisting of paper, glass, borosilicate, metal, polymer, alloy, and composite.

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13. The method according to claim 11, wherein said container is porous, permeable, or semi-permeable.

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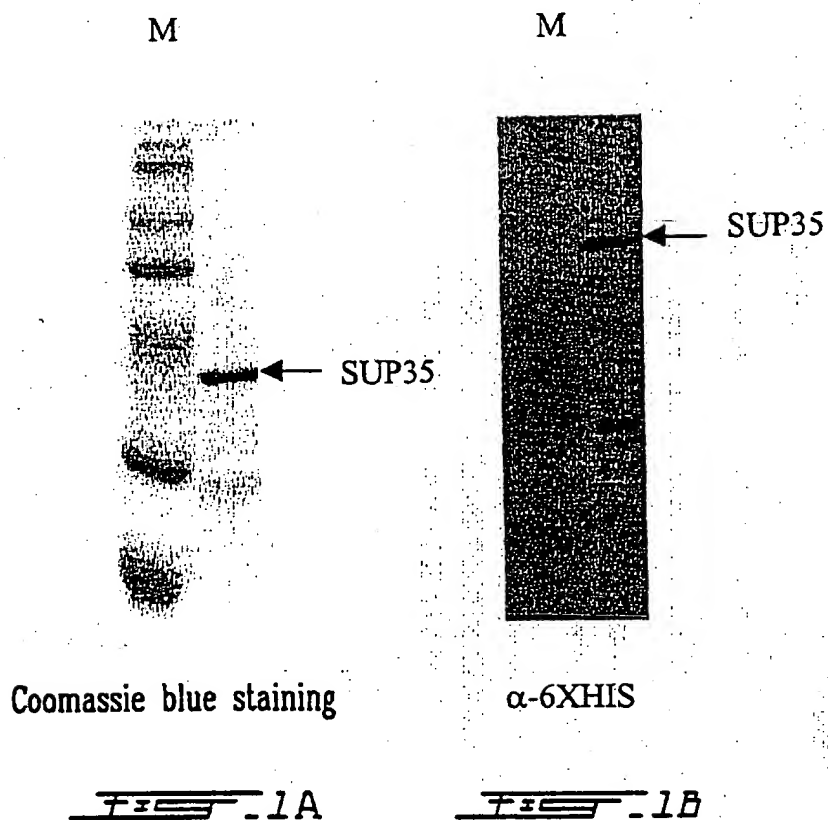
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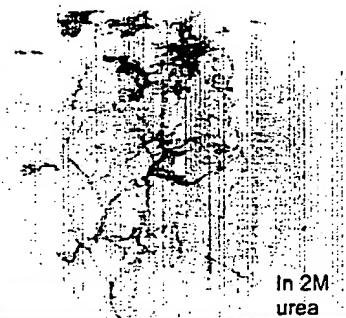
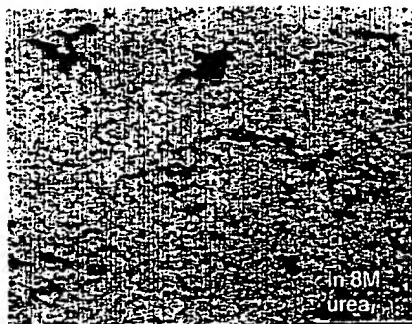
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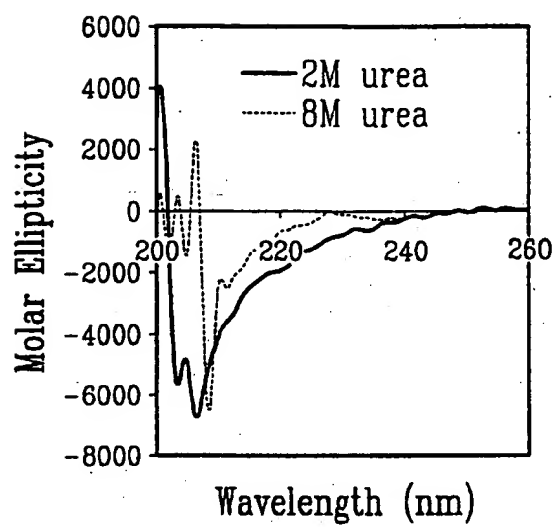
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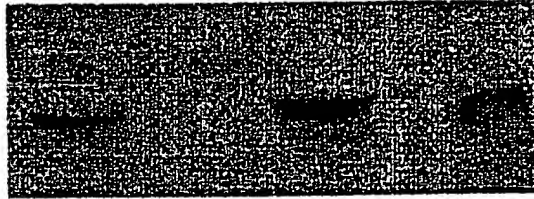
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FIG. 3

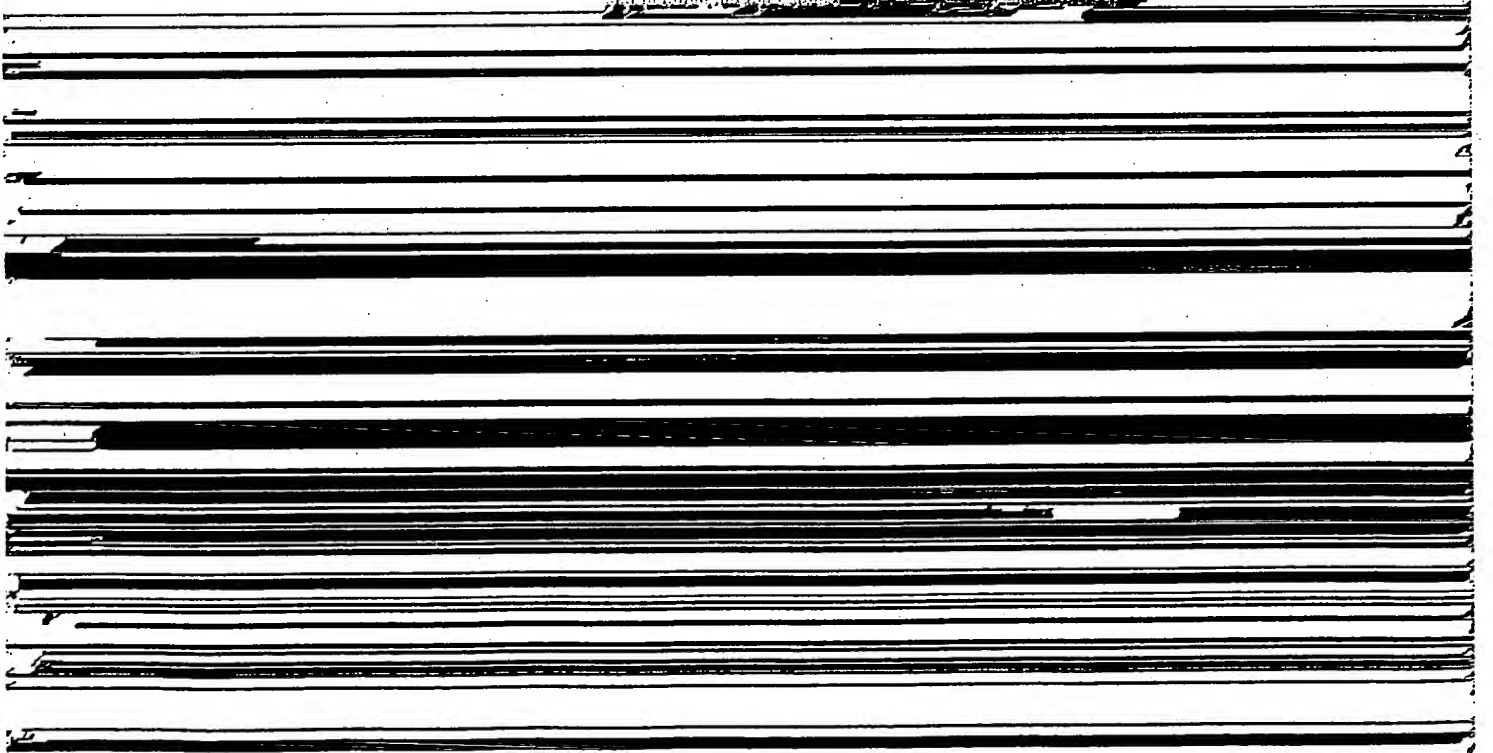
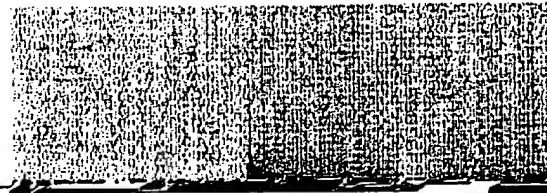
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M		M
<u>8M Urea</u>		<u>TA</u>
U T		U T

Autoclave



		<u>TA</u>	4A
<u>8M Urea</u>		<u>TA</u>	
U T		U T	



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Coomassie blue

<u>8M Urea</u>		<u>2M Urea</u>		<u>TA</u>	
U	T	U	T	T	U

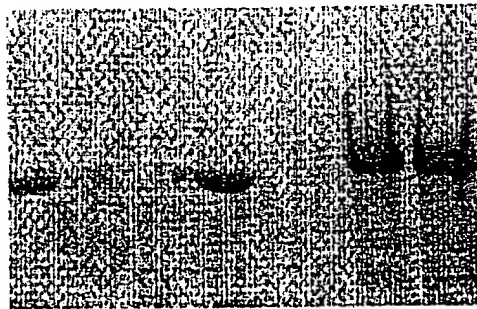


FIG. 5A

<u>2M Urea</u>		<u>TA</u>	
U	T	U	T

